

**APTAMERS TO HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR-3****STATEMENT OF GOVERNMENT SUPPORT**

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**CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims priority under Section 119(e) from U.S. Provisional Application Serial No. 60/488,679 filed July 18, 2003, the contents of which are incorporated herein by reference.

**FIELD OF THE INVENTION**

The present invention provides compositions of nucleic acid aptamers that bind human epidermal growth factor receptor-3 and methods for their use.

**BACKGROUND OF THE INVENTION**

Receptor tyrosine kinases (RTKs) are involved in a broad spectrum of cell growth and differentiation events. RTKs are classified based on sequence homology and domain organization. Type I RTKs include the epithelial growth factor receptor (EGFR) and the Human EGF Receptor homologues HER2 (HER2/neu, p185), HER3 and HER4 (also named c-erbB1-4). Overexpression of several members of this receptor family, especially EGFR and HER2, is associated with a variety of solid tumor malignancies (see, e.g. Dougall et al. (1993) J Cell Biochem 53, 61-73; Berchuck et al. (1990) Cancer Res 50, 4087-91; Schneider et al. (1989) Cancer Res 49, 4968-71; Yokota et al. (1988) Oncogene 2, 283-7; and Slamon et al. (1989) Science 244, 707-12). Overexpression of HER2 is found in 20-30% of breast cancers and results in ligand independent activation and more aggressive growth behavior (see, e.g. Slamon et al. (1989) Science 244, 707-12).

Among the four mammalian type I RTKs, HER3 is unique because of its catalytically deficient kinase domain (see, e.g. Guy et al. (1994) *Proc Natl Acad Sci U S A* 91, 8132-6), its high propensity to self-associate in the absence of ligand (see, e.g. Landgraf et al. (2000) *Biochemistry* 39, 8503-8511) and the ability of the monomeric species of HER3ECD to assume a locked conformation, using an intramolecular tether (see, e.g. Cho et al. (2002) *Science* 297, 1330-3). HER3 binds a variety of isoforms of the EGF homolog heregulin, and signaling relies on heterodimerization with other RTKs, preferentially HER2 (see, e.g. Sliwkowski et al. (1994) *Journal of Biological Chemistry* 269, 14661-5). HER2 has a potent cytoplasmic kinase domain but is deficient in ligand binding. Simultaneous overexpression of both HER2 and HER3 is found in several cancers (see, e.g. Naidu et al. (1998) *Br J Cancer* 78, 1385-90; and Krahn et al. (2001) *Eur J Cancer* 37, 251-9), and the increased drug resistance in many HER2 overexpressing cancers depends on increased levels of HER3 or EGFR (see, e.g. Chen et al. (2000) *Biochem Biophys Res Commun* 277, 757-63).

Ligand controlled signaling by type I RTKs involves receptor dimers. However, at elevated expression levels HER2 and other RTKs are likely to be engaged in a broader range of interactions. Activation of HER2 has been shown to result in the formation of large clusters of activated receptors from preexisting smaller clusters (see, e.g. Nagy et al. (1999) *J Cell Sci* 112 (Pt 11), 1733-41). For EGFR, ligand-independent interactions of receptors have been implicated in the rapid spread of signal over the entire surface of the cell after localized stimulation with immobilized ligand (see, e.g. Verveer et al. (2000) *Science* 290, 1567-70).

The extracellular domains of RTKs (ECDs) provide attractive targets for macromolecular anti-cancer drugs. Examples include soluble ECDs of the receptors (see, e.g. Azios et al. (2001) *Oncogene* 20, 5199-209) and antibodies against the ECDs (see, e.g. Ranson et al. (2002) *Oncology* 63 Suppl 1, 17-24; and Agus et al. (2002) *Cancer Cell* 2, 127-37). Herceptin, a humanized antibody against HER2, has shown great promise in the treatment of HER2 overexpressing breast cancers (see, e.g. Pegram et al. (1999) *Oncogene* 18, 2241-51), thus demonstrating two important points. First, interference by large macromolecules with this first level of the signaling cascade holds

therapeutic potential. Second, intrinsic toxicity is not required for a drug to be effective against cells that overexpress growth factor receptors.

As macromolecular drugs, RNA aptamers against RTKs have advantages over proteins. Libraries of randomized RNAs can be generated *in vitro* with a very high level of sequence complexity. Libraries can be screened *in vitro* using SELEX (Systematic Evolution of Ligands by EXponential enrichment) (see, e.g. Gold et al. (1995) *Annu Rev Biochem* 64, 763-97). A variety of chemical modifications exists for nucleic acids, such as the incorporation of radiolabels, fluorescent probes, or cross-linking reagents, and modifications to the backbone or specific bases can be introduced at will, thereby adding functionality and stability. RNA aptamers are non-immunogenic, and the use of fluorine or amino groups in the 2' position significantly enhances the half-life of RNA-aptamers in serum.

In recent years, aptamers have been selected successfully against several extracellular protein ligands, such as TGF $\beta$ , PDGF, basic FGF and VEGF (see, e.g. Golden et al. (2000) *J Biotechnol* 81, 167-78; Pietras et al. (2001) *Cancer Res* 61, 2929-34; Jellinek et al. (1995) *Biochemistry* 34, 11363-72; and Jellinek et al. (1994) *Biochemistry* 33, 10450-6). Aptamers against VEGF shrink tumors in mice and have shown promise for the treatment of macular dysfunction (see, e.g. Martin et al. (2002) *Retina* 22, 143-52; and Kim et al. (2002) *Proc Natl Acad Sci U S A* 99, 11399-404). An aptamer against the proinflammatory cytokine oncostatin M is being evaluated for use against rheumatoid arthritis (see, e.g. Rhodes et al. (2000) *J Biol Chem* 275, 28555-61), and aptamers against blood coagulation factors VIIa and IXa are under investigation as anticoagulants (see, e.g. Rusconi et al. (2000) *Thromb Haemost* 84, 841-8; and Rusconi et al. (2002) *Nature* 419, 90-4).

As a target for aptamer selection, RTKs stand out through their large size. The extracellular domains of type I RTKs are heavily glycosylated, may form several higher molecular weight complexes, and a variety of distinct conformations are likely to exist. These differences pose a considerable challenge for the application of SELEX to RTKs. HER3 exemplifies these challenges, because of its high propensity to self-associate. Consequently, there is a need in the art for methods that allow the identification

aptamers to RTKs such as HER3 as well as specific aptamers that recognize these molecules. The invention disclosed herein satisfies this need.

### **SUMMARY OF THE INVENTION**

In the invention disclosed herein, SELEX methodology was utilized to select RNA aptamers against the oligomeric states of the extracellular domains of HER3 (HER3ECD, monomeric m.w. 82,000 D). A number of specific RNA aptamers against the oligomeric states of the extracellular domains of HER3 and methods for making and using these aptamers are disclosed herein. One of the aptamers, A30, binds with high affinity to a limited number of binding sites in the oligomeric state of HER3ECD. Binding of A30 and the HER3 ligand heregulin are not competitive. Instead, the disruption of HER3 oligomers by heregulin results in an almost tenfold increase in total binding sites, but the newly created binding sites are of lower affinity. High affinity binding of A30 inhibits heregulin-dependent tyrosine phosphorylation of HER2 as well as the heregulin induced growth response of MCF7 cells. As an example of an aptamer against a large macromolecular protein complex, A30 can serve as a tool for the analysis of receptor interactions and may serve as a lead compound for the development of inhibitors against overexpressed RTKs in pathologies associated with HER3 overexpression such as cancer.

The invention disclosed herein has a number of embodiments. One embodiment of the invention is an isolated nucleic acid molecule that binds HER3 polypeptide (SEQ ID NO: 2), wherein the nucleic acid molecule comprises the sequence: 5'-CAGCGAAAGUUGCGUAUGGGUCACAUCGCAG-3' (SEQ ID NO: 19). In specific illustrative embodiments of the invention, the nucleic acid molecule comprises the sequence shown in SEQ ID NO: 7, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 or SEQ ID NO: 18. In addition, the nucleic acid molecules of the invention typically form a triple hairpin loop structure as shown in Figure 10 and further comprises a stem structure as shown in Figure 10, wherein the stem structure has at least 1, 2, 3, 4, 5 or 6 base pairings.

Optionally, a nucleic acid molecule of the invention is contained within a pharmaceutical composition, for example a pharmaceutical carrier, excipient or stabilizer. In certain embodiments of the invention, the nucleic acid molecule can be labeled with a detectable marker. Other embodiments of the invention include a vector comprising the nucleic acid molecules of the invention, for example DNA vectors (wherein thymidine (T) replaces uridine (U)) and/or host cells comprising such vectors.

Embodiments of the invention include a variety of methods for using the disclosed nucleic acid molecules for example as probes for HER3 polypeptides. One typical embodiment is a method of binding a nucleic acid molecule comprising the sequence 5'-CAGCGAAAGUUGCGUAUGGGUCACAUCGCAG-3' (SEQ ID NO: 19) to a HER3 polypeptide encoded by a polynucleotide of SEQ ID NO: 1 comprising combining the nucleic acid molecule and the HER3 polypeptide for a time and under conditions effective to allow the nucleic acid molecule to bind to the HER3 polypeptide such that said binding occurs. In certain embodiments of such methods, the nucleic acid molecule and the HER3 polypeptide are combined in vitro (e.g. in a patient biopsy sample). Alternatively, the nucleic acid molecule and the HER3 polypeptide are combined in vivo (e.g. in a therapeutic regimen that treats a patient suffering from a pathology characterized by a dysregulation of a biological pathway associated with HER3, HER2 and/or heregulin). Embodiments of the invention can include additional methodological steps such as examining the HER3 polypeptide for evidence of said binding via protocols such as a native gel mobility shift assay. Optionally, the nucleic acid molecule is labeled with a detectable marker.

In certain embodiments of the invention, the methods include examining the affinity of the nucleic acid molecule for the HER3 polypeptide and/or the number of binding sites for the nucleic acid molecule present on the HER3 polypeptide. In an illustrative embodiment of the invention, the nucleic acid molecule is combined with HER3 polypeptide expressed on the surface of a human cell and the method further comprises the step of examining the affinity of the nucleic acid molecule for the HER3 polypeptide. In yet another embodiment of the invention, the nucleic acid molecule is combined with HER3 polypeptide expressed on the surface of a human cell and the

method further comprises the step of examining the number of nucleic acid molecule binding sites in the HER3 polypeptide.

Alternative embodiments of the invention can include additional methodological steps such as examining the HER3 polypeptide for evidence of said binding via protocols which examine the HER3 polypeptide and/or the modulation of one or more activities of the biological pathway associated with HER3, HER2 and/or heregulin. In one embodiment, the nucleic acid molecule is combined with HER3 polypeptide expressed on the surface of a human cell that further expresses HER2 polypeptide (SEQ ID NO: 6) and the method further comprises examining the human cell for evidence of said binding, wherein the inhibition of heregulin (SEQ ID NO: 4) induced tyrosine phosphorylation of HER2 in the human cell provides evidence of said binding. In another embodiment, the nucleic acid molecule is combined with HER3 polypeptide expressed on the surface of a human cell that further expresses HER2 polypeptide (SEQ ID NO: 6) and the method further comprises examining the human cell for evidence of said binding, wherein the inhibition of heregulin (SEQ ID NO: 4) induced growth in the human cell provides evidence of said binding.

Another typical embodiment of the invention is a method of modulating heregulin mediated signalling in a mammalian cell, wherein the cell expresses a HER2/HER3 complex on the surface of the cell, the method comprising contacting the cell with an aptamer polynucleotide disclosed herein under conditions that allow the aptamer polynucleotide to interact with an extracellular portion of a HER3 polypeptide expressed by the cell so that heregulin mediated signalling in the mammalian cell is modulated. In a specific embodiment of the invention, the modulation of heregulin mediated signalling in a mammalian cell comprises an inhibition of heregulin mediated signalling (e.g. using the A30 aptamer). In an alternative embodiment, the modulation of heregulin mediated signalling in a mammalian cell comprises an enhancement of heregulin mediated signalling (e.g. using the A18 aptamer). In preferred embodiments, the modulation of heregulin mediated signalling in a mammalian cell comprises an inhibition of heregulin mediated signalling the mammalian cell is a human breast cancer or ovarian cancer cell.

The invention disclosed herein further provides articles of manufacture and kits which include reagents for performing for example, the methods disclosed herein. One illustrative embodiment is a kit comprising a nucleic acid molecule comprising the sequence 5'-CAGCGAAAGUUGCGUAUGGGUCACAUCGCAG-3' (SEQ ID NO: 19) and methods for its use.

### **BRIEF DESCRIPTION OF THE FIGURES**

**Figure 1:** Design of aptamers and sequences of six selected aptamers with affinity for HER3ECD. The initial aptamer library was created by PCR of the indicated DNA template, containing a randomized core of 49 nucleotides (SEQ ID NO: 14). The primers for the PCR are indicated underneath the template. The aligned sequences represent the randomized core of six of the 29 clones that were selected based on robust binding in gel shift assays with HER3ECD. For example, the 49 nucleotide A6 aptamer core sequence within the indicated DNA template has the sequence 5'-TAATACGACTCACTATAGGGAATTCCGCGTGTGCAGAACAAATCGCATAGGC CGCAAGGTTAGTTTCGTTGTCCGCCCGGTGCAGTCCGTTCTGGGATCCTC-3' (SEQ ID NO: 20, as this is described as a DNA template, "U" is therefore replaced with "T"). A6, A18, A19, A23, A30 and A37 in this figure correspond to SEQ ID NOS: 8-13 respectively. The same six aptamers were used for the inhibition studies, shown in Figure 2.

**Figure 2:** Screening of selected aptamers for their inhibition of biological activities associated with HER2 activation by heregulin. MCF7 cells were either stimulated with 10 nM wild type heregulin (hrg) or low affinity heregulin (la-hrg) in the presence of 100 nM of the various aptamers indicated. Tyrosine phosphorylation of HER2 was determined by Western blotting. The numbers above each lane indicate aptamer clones. While A30 reduces tyrosine phosphorylation almost to the level observed for the unstimulated control (Ctrl), other aptamers, such as A6 and A18, enhance the activation by low affinity heregulin. The level of tyrosine phosphorylation in the presence of the various aptamers is shown in column format to the right, relative to the uninhibited stimulation with la-hrg (-). The differences in tyrosine phosphorylation of

HER2 are not due to changes in the levels of HER2, visualizes by direct detection of the receptor.

**Figure 3:** Native gel mobility shift assay of radiolabeled A30. Radiolabeled A30 (alone = Ctrl) is shifted to several slower migrating species in the presence of purified HER3ECD (0.5  $\mu$ M). This interaction does not occur with HER2-ECD or heregulin, both 0.5  $\mu$ M, and is not inhibited by an excess of tRNA (4  $\mu$ M).

**Figure 4:** Binding of A30 to HER3ECD and cellular HER3. A) Binding of A30 to purified HER3ECD, as determined by the replacement of radiolabeled A30 by unlabeled A30 in a gel-shift analysis, indicates a small number of high affinity binding sites in the absence of heregulin ( $\circ$ ). Addition of heregulin ( $\bullet$ ) generates additional binding sites of lower affinity. B) Binding of A30 to cellular HER3 also shows an increase in binding sites upon addition of heregulin ( $\bullet$ ), though less pronounced than in solution. Binding data are shown as displaced labeled A30 (cpm) as a function of unlabeled A30. C) The Scatchard analysis of data shown in B) reveals a smaller increase in high affinity binding sites parallel with the larger increase in low affinity sites after addition of heregulin ( $\bullet$ ). C in this analysis represents displaced cpm, L represents the concentration of unlabeled A30 (nM).

**Figure 5:** Gelshift pattern of A30 and HER3ECD in the presence and absence of heregulin and competition with unlabeled A30. The binding of radiolabeled A30 to HER3ECD (0.5  $\mu$ M) is shown at various concentration of total A30 (shown in  $\mu$ M). Addition of a molar excess of heregulin (lanes 5-7) enhances binding, but does not qualitatively change the pattern of shifted bands (A, B, C).

**Figure 6:** Gelshift of radiolabeled A30 and direct visualization of HER3ECD at elevated protein concentrations. Radiolabeled A30 (0.3 nM) is present in all lanes. The concentration of HER3ECD is 2  $\mu$ M and heregulin (6  $\mu$ M) and unlabeled A30 (8  $\mu$ M) were added at a molar excess as indicated above the gel. Gels were either analyzed by direct coomassie staining (CB) or autoradiography ( $^{32}$ P) as indicated. On this 4-15% Phast gel, both heregulin and A30 run with the running front.

**Figure 7:** Inhibition of HER2/HER3 activation by A30. A) Anti-phosphotyrosine Western blot of MCF7 cell lysates after HER2 immunoprecipitation.



Cells were stimulated with wild type heregulin (5nM) unless indicated otherwise (-). A30 was added at the concentrations indicated above each lane. The differences in tyrosine phosphorylation of HER2 are not due to changes in the levels of HER2, visualizes by direct detection of the receptor. B) Tyrosine phosphorylation of HER2 following stimulation with EGF is not inhibited by A30. EGF or heregulin were added to MCF7 cells at 5nM in the presence or absence of A30 (300 nM).

**Figure 8:** Inhibition of heregulin induced cell growth in MCF7 cells. Cell growth (OD 560 after MTT assay) was measured as a function of A30 concentration (nM) in the presence of two different concentrations of wild type heregulin (no heregulin (▲), 5 nM (□) and 100 nM heregulin (●).

**Figure 9:** A comparison of various A30 embodiments of the invention (from top to bottom: SEQ ID NOS: 15, 16, 17, 12 and 7) as well as an A30 consensus sequence (SEQ ID NO: 18) and the A30 minimal loop sequence (SEQ ID NO: 19).

**Figures 10A and 10B:** Hairpin loop and stem structures formed by A30 embodiments of SEQ ID NO: 16 and SEQ ID NO: 7 respectively. Both A30 embodiments of SEQ ID NO: 7 and SEQ ID NO: 16 exhibit a triple hairpin loop structure formed by the nucleotides identified in SEQ ID NO: 19. The A30 embodiment shown in SEQ ID NO: 7 comprises a stem structure having 6 base pairings.

### **DETAILED DESCRIPTION OF THE INVENTION**

Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized molecular cloning methodologies

described in Sambrook and Russel, 2001, Molecular Cloning: A Laboratory Manual, 3d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y and Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995). As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined protocols and/or parameters unless otherwise noted.

## I. DEFINITIONS

As used herein, the term "polynucleotide" means a polymeric form of nucleotides of at least 10 bases or base pairs in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide, and is meant to include single and double stranded forms of DNA and/or RNA. In the art, this term is often used interchangeably with "oligonucleotide". A polynucleotide can comprise a nucleotide sequence disclosed herein wherein thymidine (T) can also be uracil (U); this definition pertains to the differences between the chemical structures of DNA and RNA, in particular the observation that one of the four major bases in RNA is uracil (U) instead of thymidine (T).

As used herein, the term "polypeptide" means a polymer of at least about 4, 5, 6, 7, or 8 amino acids. Throughout the specification, standard three letter or single letter designations for amino acids are used. In the art, this term is often used interchangeably with "peptide" or "protein".

"Isolated," when used to describe the various molecules disclosed herein, means for example a polynucleotide molecule that has been identified and separated and/or recovered from a component of the environment in which the molecule is produced. Contaminant components of this environment are materials that would typically interfere with diagnostic or therapeutic uses for the polynucleotides of the invention, and may include polypeptides and polynucleotides, and other proteinaceous or non-proteinaceous solutes.

"Growth inhibition" when used herein refers to the growth inhibition of a cell *in vitro* and/or *in vivo*. The inhibition of cell growth can be measured by a wide variety of methods known in the art including those described in the Examples below. A "growth

inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell *in vitro* and/or *in vivo*. Thus, a growth inhibitory agent may be one which significantly reduces the percentage of cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), TAXOL®, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in The Molecular Basis of Cancer, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13.

"Biologically active" or "biological activity" for the purposes herein means (a) having the ability to modulate the activity and/or function of a polypeptide such as heregulin, HER2 and/or HER3; and/or (b) having the ability to modulate the growth of at least one type of mammalian cancer cell or experimentally transformed cell *in vivo* or *ex vivo*.

The terms "agonist" and "agonistic" when used herein refer to a molecule which is capable of, directly or indirectly, substantially inducing, promoting or enhancing biological activity or activation of a molecule such as heregulin, HER2 or HER3. The term "antagonist" when used herein refers to a molecule which is capable of, directly or indirectly, substantially inhibiting the biological activity or activation of a molecule such as heregulin, HER2 or HER3.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (*e.g.* At<sup>211</sup>, I<sup>131</sup>, I<sup>125</sup>, Y<sup>90</sup>, Re<sup>186</sup>, Re<sup>188</sup>, Sm<sup>153</sup>, Bi<sup>212</sup>, P<sup>32</sup> and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of conditions like cancer. Examples of chemotherapeutic agents include alkylating agents alkyl sulfonates such as busulfan; aziridines such as benzodopa, anti-metabolites such as methotrexate; folic acid analogues such as denopterin; taxoids, *e.g.* paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, NJ) and doxorubicin (DOXIL®, Rhône-Poulenc Rorer, Antony, France); platinum analogs such as cisplatin; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are heregulin, growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-alpha and -beta; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-alpha; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, -beta and -gamma colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; a tumor necrosis factor such as TNF-alpha or TNF-

beta; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

"Treatment" or "therapy" refer to both therapeutic treatment and prophylactic or preventative measures.

The term "therapeutically effective amount" refers to an amount of a drug effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the disorder. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy *in vivo* can, for example, be measured by assessing tumor burden or volume, the time to disease progression (TTP) and/or determining the response rates (RR).

"Mammal" for purposes of treatment or therapy refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, *etc.* Preferably, the mammal is human.

The terms "cancer", "cancerous", or "malignant" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include breast cancer, ovarian cancer, colon cancer, colorectal cancer, rectal cancer, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, Hodgkin's and non-Hodgkin's lymphoma, testicular cancer, esophageal cancer, gastrointestinal cancer, renal cancer, pancreatic cancer, glioblastoma, cervical cancer, glioma, liver cancer, bladder cancer, hepatoma, endometrial carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer. Diagnosis in mammals of the various pathological

conditions described herein can be made by the skilled practitioner. Diagnostic techniques are available in the art which allow, e.g., for the diagnosis or detection of cancer or immune related disease in a mammal. For instance, cancers may be identified through techniques, including but not limited to, palpation, blood analysis, x-ray, NMR and the like.

## II. CHARACTERIZATION OF ASPECTS OF THE INVENTION

The disclosure provided herein reports the successful selection of aptamers specific for the extracellular domains of HER3. SELEX requires a high abundance of target and selection against proteins the size of HER3ECD creates special challenges. At elevated concentrations HER3ECD exists in an oligomeric form. This represents, to our knowledge, the largest target for successful aptamer selection to date. Some observed differences to "more conventional" SELEX may therefore be a reflection of the large size of the target surface. Most notably, our selection resulted in a family of aptamers without apparent consensus. At least two classes of aptamers were apparent, causing either inhibition or enhancement of heregulin dependent activation. Those two classes are represented by A30 and A18 respectively, which showed no mutual competition for binding in gel-shift studies. These findings provide evidence that the lack of sequence convergence is at least in part a reflection of selection against different binding sites.

The selected aptamers show high specificity for HER3ECD. We analyzed A30 in more detail. Despite a high level of homology between the ECDs of HER2 and HER3, A30 shows no binding to the ECD of HER2 or to heregulin, even at concentrations far above those used in inhibition studies (Fig. 3). Most aptamers obtained during the selection show no binding to HER3ECD, providing a randomized control set for unspecific binding. Non-specific RNA (Fig. 3) as well as aptamer with independent binding to HER3ECD (A18) do not interfere with the binding of A30 to HER3ECD.

On a cellular level, we cannot exclude the possibility of additional low affinity targets. However, the inhibitory properties of A30 are directly linked to the action of heregulin. A30 does not exhibit general growth inhibition but specifically inhibits the growth stimulatory component elicited by heregulin. While heregulin-induced tyrosine

phosphorylation of HER2, which proceeds primarily through HER2-HER3 complexes, is inhibited, the activation of HER2 by EGF, requiring dimers of EGFR-HER2, is not inhibited.

The inhibitory properties of A30 raise the question about possible modes of binding and the method by which signaling is inhibited. At present, we have no information on the localization of the A30 binding site on the HER3ECD. However, the fact that A30 binding is not competitive with heregulin and the size of the aptamer make it less likely that domains 1-3 of the ECD, involved in ligand binding, are the target. Recent models of HER3 activation, based on the crystal structure of the HER3ECD and ECD of EGFR with bound ligand (see, e.g. Cho et al. (2002) *Science* 297, 1330-3; and Ogiso et al. (2002) *Cell* 110, 775-87), assume receptor interactions in domains 2 and 4 in the activated complex of HER2 and HER3 (see, e.g. Schlessinger, J. (2003) *Science* 300, 750-2). At present, domain 4 appears to be the most likely target for A30 and aptamer binding is therefore anticipated to interfere with the dimerization of HER2 and HER3 but not heregulin binding. In the crystal structure, domains 2 and 4 of HER3ECD are also involved in an intramolecular "lock".

The analysis of the mode of binding of A30 to HER3ECD shows the presence of at least two different modes of interaction, evident by the presence of multiple gelshifted species (Figures 3, 5 and 6). Without being bound by a specific scientific theory, the current working model for the effect of heregulin on A30 binding assumes that A30 preferentially binds oligomers of HER3ECD. Given that aptamers were initially selected at high concentrations of HER3ECD, oligomers of HER3 would have been the primary target of selection. This hypothesis is also consistent with several experimental findings. At high concentrations of HER3ECD (Figure 6), disruption of oligomers by excess heregulin is incomplete. Under those conditions of partial disruption of oligomers and an excess of oligomers over A30 (Figure 6, lane 8), oligomers of HER3ECD are the preferred binding site for A30. However, the number of accessible sites in the oligomeric species appears to be limited. Based on this model, the heregulin-induced increase in A30 binding sites at lower concentrations of HER3ECD would reflect the more complete disruption of oligomers by heregulin,

resulting in increased access for A30 to the ECD but the resulting binding sites have reduced affinity. One possible explanation for the different interaction with oligomers and monomers assumes a binding surface on the oligomers that contains residues from adjacent ECD molecules at the periphery of ECD clusters. A disruption of oligomers would make individual ECDs more accessible but part of the binding interface would be lost, resulting in weaker binding. Further, support for the notion of substoichiometric binding of A30 to HER3 oligomers comes also from the fact that the maximum number of cellular binding sites for A30 ( $3250 \pm 200$ ) falls short of the total number of HER3 receptors (25,000) in MCF7 cells. This discrepancy is partially reduced by the addition of heregulin. This partial disruption of HER3 oligomers on the cell surface is consistent with the partial disruption of high concentrations of soluble ECDs in solution, even in the presence of excess heregulin.

If A30 is in fact capable of binding to HER3ECD monomers, albeit with lower affinity, a large excess of A30 should disrupt oligomers of HER3ECD. Evidence for such a disruption is provided in Figure 6 (lane 2 versus 4). In our model, based on a transition of HER3ECD oligomers to monomers, (species a, lane 8 and 9 of Figure 6) would represent the binding of a single A30 to the oligomer under conditions of a large excess of oligomeric HER3ECD. High concentrations and a molar excess of A30 over HER3ECD would stabilize the monomeric form of HER3ECD and would also result in complete saturation of all accessible binding sites on the remaining oligomer. The high negative net charge of such a complex should result in a significantly shifted oligomeric species. Assuming that species b represents such a shifted oligomer while species c represent a monomer complex, the low ratio of A30 per HER3ECD (Figure 6, lane 2) is consistent with a substoichiometric number of accessible sites on the oligomer.

While not being bound by a specific scientific theory, the above model provides a simple working hypothesis for the mechanism of A30 binding to HER3ECD. The model does not account for the double band for species c (Fig. 6, lane 1 and 2) and species C in Figure 5. The crystal structure of HER3ECD suggests two distinct conformations of HER3ECD (open and locked). Such differences in conformation could contribute to differences in migration of A30-monomer complexes. Also,



alternative explanations, such as species with a different stoichiometry of A30 binding to HER3ECD monomers, cannot be ruled out at this point.

Regardless of the complexities of the mechanism of binding by A30, the data demonstrate that the selected aptamer is specific for HER3ECD and inhibits the heregulin-induced activation of HER3/HER2 (Fig. 7). The lack of competition between heregulin and A30 indicates that the inhibitory effect of A30 on tyrosine phosphorylation is not due to inhibition of heregulin binding. The activity of A30 and similar aptamers against HER3 may complement antibodies that target HER2, especially in cases where elevated levels of HER3 enhance the effect of HER2 overexpression.

We have generated an aptamer against the extracellular domains of HER3. The ease with which aptamers can be chemically modified makes them ideal starting points for the synthesis of a broad range of biochemical tools that may shed light into the complex interactions between receptor tyrosine kinases in a membrane setting. Given that A30 has not been subjected to further modifications that could enhance its serum stability and binding affinity, it already shows a remarkably strong inhibitory effect on heregulin-induced growth stimulation of MCF7 cells. The mechanism of inhibition by A30 is likely to be complex and requires further analysis. Additional contributions beyond the direct targeting of HER3 cannot be ruled out at this point. However, the inhibitory properties of A30 demonstrate its potential usefulness as a lead compound for the design of anticancer drugs. The activity of A30 is of special importance in light of the paradigm established by Herceptin. Herceptin has demonstrated that a non-toxic and non-membrane permeating macromolecule has the potential to be a potent reagent in the treatment of cancer. Anti-HER3ECD aptamers, in isolation or combination with other treatments such as anti-HER2 antibodies or anti-HER3 aptamers that bind different binding sites, may therefore become a valuable addition to the repertoire of inhibitors that target cancers that overexpress HER2.

The invention provides methods for modulating HER3, and/or heregulin activity in mammalian cells which comprise exposing the cells to a desired amount of HER3 aptamer that affects heregulin mediated HER3 activation. Preferably, the amount of HER3 aptamer employed will be an amount effective to affect the binding and/or activity of the respective

ligand or respective receptor to achieve a therapeutic effect. This can be accomplished *in vivo* or *ex vivo* in accordance, for instance, with the methods described below. Exemplary conditions or disorders to be treated with HER3 aptamers include cancer such as those associated with an aberrant expression of HER2, HER3 and/or heregulin. In particular, the molecules described herein are useful in treating various pathological conditions associated with the dysregulation of the biological activities associated with heregulin, HER2 and/or HER3, such as cancer (see, e.g. Stove et al., J Invest Dermatol. 2003 Oct;121(4):802-12; Earp et al., Trans Am Clin Climatol Assoc. 2003;114:315-33; Mendoza et al., Cancer Res. 2002 Oct 1;62(19):5485-8; Kumar et al., Semin Oncol. 2001 Oct;28(5 Suppl 16):27-32; Schelfhout et al., J Natl Cancer Inst. 2000 Apr 19;92(8):622-8; Gilbertson et al., Cancer Res. 1997 Aug 1;57(15):3272-80; Gullick Cancer Surv. 1996;27:339-49; and Wallasch et al., EMBO J. 1995 Sep 1;14(17):4267-75). These conditions can be treated by modulating a selected activity associated with heregulin mediated activation of the HER3 associated receptor complex in a mammal through, for example, administration of one or more HER3 aptamers described herein.

The HER3 aptamers can be administered in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Optionally, administration may be performed through mini-pump infusion using various commercially available devices. Effective dosages and schedules for administering antagonists or agonists may be determined empirically, and making such determinations is within the skill in the art. Single or multiple dosages may be employed. Interspecies scaling of dosages can be performed in a manner known in the art, e.g., as disclosed in Mordenti et al., Pharmaceut. Res., 8:1351 (1991).

As noted above, the HER3 aptamers useful in the methods of the invention can be incorporated into pharmaceutical compositions suitable for administration into a mammal. Such compositions typically comprise at least one HER3 aptamer and a pharmaceutically acceptable carrier. Methods for formulating the HER3 aptamer compounds of the invention for pharmaceutical administration are known to those of

skill in the art. See, for example, Remington: The Science and Practice of Pharmacy, 19<sup>th</sup> Edition, Gennaro (ed.) 1995, Mack Publishing Company, Easton, PA.

As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, such media can be used in the compositions of the invention. Supplementary active compounds can also be incorporated into the compositions. A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration.

The pharmaceutical compositions of the invention, comprising HER3 aptamers, are administered in therapeutically effective amounts. The "therapeutically effective amount" refers to a nontoxic dosage level sufficient to induce a desired biological result (e.g. a diminution of the severity of the symptoms associated with a pathological condition such as breast or ovarian cancer). Amounts for administration may vary based upon the desired activity, the diseased state of the mammal being treated, the dosage form, method of administration, patient factors such as age, sex, and severity of disease. It is recognized that a therapeutically effective amount is provided in a broad range of concentrations. Such range can be determined based on in vitro and/or in vivo assays.

Therapeutic compositions of the HER3 aptamers can be prepared by mixing the desired molecule having the appropriate degree of purity with optional pharmaceutically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, 16th edition, Osol, A. ed. (1980)), in the form of lyophilized formulations, aqueous solutions or aqueous suspensions. Acceptable carriers, excipients, or stabilizers are preferably nontoxic to recipients at the dosages and concentrations employed, and include buffers such as Tris, HEPES, PIPES, phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl

paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; and/or non-ionic surfactants such as TWEEN<sup>TM</sup>, PLURONICS<sup>TM</sup> or polyethylene glycol (PEG).

Additional examples of such carriers include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts, or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, and cellulose-based substances. Carriers for topical or gel-based forms include polysaccharides such as sodium carboxymethylcellulose or methylcellulose, polyvinylpyrrolidone, polyacrylates, polyoxyethylene-polyoxypropylene-block polymers, polyethylene glycol, and wood wax alcohols. For all administrations, conventional depot forms are suitably used. Such forms include, for example, microcapsules, nano-capsules, liposomes, plasters, inhalation forms, nose sprays, sublingual tablets, and sustained-release preparations.

Solutions or suspensions used for administering HER3 aptamers can include the following components: a sterile diluent such as water for injection, saline solution; fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. In one embodiment, a pharmaceutical composition can be delivered via slow release formulation or matrix comprising HER3 aptamers or DNA constructs suitable for expression of HER3 aptamer in or around a site within the body.

HER3 aptamers can also be administered in the form of a variety of sustained-release preparations. For example, HER3 aptamers may be delivered to the lung for slow release via encapsulation or carrier materials such as liposomes, or other drug "shells" such as albumin (Albunex by Molecular Biosystems), sugars (Levovist by Schering), gelatins, or lipids. Other suitable examples of sustained-release preparations for use with polypeptides including semipermeable matrices of solid hydrophobic polymers containing the protein, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (*e.g.*, poly(2-hydroxyethyl-methacrylate) as described by Langer *et al.*, J. Biomed. Mater. Res., **15**: 167-277 (1981) and Langer, Chem. Tech., **12**: 98-105 (1982) or poly(vinylalcohol)), polylactides (U.S. Patent No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, Biopolymers, **22**: 547-556 (1983)), non-degradable ethylene-vinyl acetate (Langer *et al.*, *supra*), degradable lactic acid-glycolic acid copolymers such as the Lupron Depot (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

The route of administration may vary depending on the desired effect and/or outcome. Generally for modulation of an HER3 mediated response, introduction of the HER3 aptamer at or near the desired site of response is utilized. Alternatively additional routes of administration, such as a systemic administration of HER3 aptamers, may be employed. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, intramuscular, subcutaneous, oral (*e.g.*, inhalation) transdermal (topical), transmucosal (*e.g.* a nasal spray), and rectal administration. The HER3 aptamer may also be administered by perfusion techniques, such as isolated tissue perfusion, to exert local therapeutic effects. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution; fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as acetates, citrates or phosphates and agents for the adjustment of

tonicity such as sodium chloride or dextrose. Regimens of administration may vary. A single dose or multiple doses of the agent may be used. Such regimens can vary depending on the severity of the disease and the desired outcome. Following administration of a HER3 aptamer to the mammal, the mammal's physiological condition can be monitored in various ways well known to the skilled practitioner familiar with the pathological condition to be treated (e.g. breast or ovarian cancer).

It is contemplated that yet additional therapies may be employed in the methods. The one or more other therapies may include but are not limited to, administration of radiation therapy, cytokine(s), growth inhibitory agent(s), chemotherapeutic agent(s), cytotoxic agent(s), tyrosine kinase inhibitors, ras farnesyl transferase inhibitors, angiogenesis inhibitors, and cyclin-dependent kinase inhibitors which are known in the art. In addition, therapies based on therapeutic antibodies that target tumor antigens such as Rituxan<sup>TM</sup> or Herceptin<sup>TM</sup> as well as anti-angiogenic antibodies such as anti-VEGF.

Preparation and dosing schedules for chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *Chemotherapy Service* Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992). The chemotherapeutic agent may precede, or follow administration of, e.g. an aptamer, or may be given simultaneously therewith. The aptamer, for instance, may also be combined with an anti-oestrogen compound such as tamoxifen or an anti-progesterone such as onapristone (see, EP 616812) in dosages known for such molecules.

### III. TYPICAL EMBODIMENTS OF THE INVENTION

The invention disclosed herein has a number of embodiments. As is shown in Figure 10, the A30 aptamer has a number of embodiments, all of which comprise a common nucleotide sequence. Without being bound by a specific scientific theory, it is believed that this common sequence allows the formation a conformation that is associated with aptamer binding. In this context, one embodiment of the invention is an isolated nucleic acid molecule that binds HER3 polypeptide (SEQ ID NO: 2), wherein the nucleic acid molecule comprises the common sequence:

5'-CAGCGAAAGUUGCGUAUGGGUCACAUCGCAG-3' (SEQ ID NO: 19). In specific illustrative embodiments of the invention, the nucleic acid molecule comprises the specific sequence shown in SEQ ID NO: 7, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 or SEQ ID NO: 18. Typically, a nucleic acid molecule of the invention is at least 50 to 100 nucleotides in length. In addition, the nucleic acid molecules of the invention typically form a triple hairpin loop structure as shown in Figure 10 and further comprises a stem structure as shown in Figure 10 and having at least 1, 2, 3, 4, 5 or 6 base pairing (e.g. A:U or G:C).

Optionally, a nucleic acid molecule of the invention is contained within a pharmaceutical composition, for example a pharmaceutical carrier, excipient or stabilizer. In certain embodiments of the invention, the nucleic acid molecule can be labeled with a detectable marker. For example, a nucleic acid molecule probe can be labeled with a detectable marker, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator or enzyme. Other embodiments of the invention include a vector comprising the nucleic acid molecules of the invention, for example DNA vectors (wherein thymidine (T) replaces uridine (U)) and/or host cells comprising such vectors.

The RNA aptamers disclosed herein provide a tools for use for example in diagnostic assays designed to detect HER3 in a biological sample. As HER 3 polypeptide is known to be overexpressed in a variety of pathological conditions (e.g. colorectal and breast cancers), HER3 protein expression can serve a prognostic factor in the characterization of these syndromes (see, e.g. Kapitanovic et al., J. Cancer Res. Clin. Oncol. 2000, 126(4): 205-211; and Witton et al., J. Pathol. 2003, 200(3): 290-297). Consequently, diagnostic assays which examine HER3 protein expression can be used in clinical contexts pertaining to the diagnostic, prognostic and therapeutic considerations for patients suffering from diseases characterized by an aberrant expression of this molecule.

In addition, RNA aptamers disclosed herein provide a tools for use in diagnostic assays designed to detect an interaction between HER3 and HER2 in a biological sample. As is known in the art, HER2 and HER3 interact to form a complex that triggers a

signalling pathway that is known to be dysregulated in a number of pathological conditions including a variety of cancers. Consequently, diagnostic assays which allow medical practitioners to examine the status of these crucial elements within this pathway can provide information that these practitioners can use for example to both confirm the diagnosis of a specific type of pathology as well as to determine the prognostic implications of such diagnoses in an individual having a specific type of pathology.

Profiling experiments designed to characteristic patterns of protein association (e.g. HER2/HER3 oligomerization) that are associated with pathological phenotypes can further be used to determine the optimal therapeutic regimen for treating pathologies exhibiting that pattern of protein association. In particular, the HER2 polypeptide and its associated biological pathways are well known targets for a variety of therapeutic agents. As the association of HER2 and HER3 is a crucial event for the activity of a functioning pathway, assays which examine the status of this association allow practitioners to assess the likely biological effect that a specific therapeutic agent or agents which target this pathway will have in a patient suffering from a pathology characterized by a dysregulation of this HER2/HER3 signalling pathway. See, e.g. Mass, *Int. J. Radiat. Oncol. Biol. Phys.* 2002, 58(3): 932-940; Anido et al., *Clin. Cancer Res.* 2003, 9(4): 1274-1283; and Azios et al., *Oncogene*, 2001, 20(37): 5199-5209.

In view of the above, embodiments of the invention include a variety of methods for using the disclosed nucleic acid molecules. One typical embodiment is a method of binding a nucleic acid molecule comprising the sequence 5'-CAGCGAAAGUUGCGUAUGGGUCACAUCGCAG-3' (SEQ ID NO: 19) to a HER3 polypeptide encoded by a polynucleotide of SEQ ID NO: 1 comprising combining the nucleic acid molecule and the HER3 polypeptide for a time and under conditions effective to allow the nucleic acid molecule to bind to the HER3 polypeptide such that said binding occurs. As explicitly disclosed herein, the HER3 polypeptide encoded by a polynucleotide of SEQ ID NO: 1 can be a full length HER3 polypeptide as occurs for example on the surface of mammalian cells (see, e.g. Example 5 below) or alternatively can be less than full length such as the HER3ECD used to select the aptamers (see, e.g. Example 2 below). In certain embodiments of such methods, the nucleic acid molecule



and the HER3 polypeptide are combined in vitro (e.g. in a patient biopsy sample). Alternatively, the nucleic acid molecule and the HER3 polypeptide are combined in vivo (e.g. in a therapeutic regimen that treats a patient suffering from a pathology characterized by a dysregulation of a biological pathway associated with HER3, HER2 and/or heregulin). Embodiments of the invention can include additional methodological steps such as examining the HER3 polypeptide for evidence of said binding via protocols such as a native gel mobility shift assay. Optionally, the nucleic acid molecule is labeled with a detectable marker.

In certain embodiments of the invention, the methods include examining the affinity of the nucleic acid molecule for the HER3 polypeptide and/or the number of binding sites for the nucleic acid molecule present on the HER3 polypeptide. In an illustrative embodiment of the invention, the nucleic acid molecule is combined with HER3 polypeptide expressed on the surface of a human cell and the method further comprises the step of examining the affinity of the nucleic acid molecule for the HER3 polypeptide. In yet another embodiment of the invention, the nucleic acid molecule is combined with HER3 polypeptide expressed on the surface of a human cell and the method further comprises the step of examining the number of nucleic acid molecule binding sites in the HER3 polypeptide.

Alternative embodiments of the invention can include additional methodological steps such as examining the HER3 polypeptide for evidence of said binding via protocols which examine the HER3 polypeptide and/or the modulation of one or more activities of the biological pathway associated with HER3, HER2 and/or heregulin. In one embodiment, the nucleic acid molecule is combined with HER3 polypeptide expressed on the surface of a human cell that further expresses HER2 polypeptide (SEQ ID NO: 6) and the method further comprises examining the human cell for evidence of said binding, wherein the inhibition of heregulin (SEQ ID NO: 4) induced tyrosine phosphorylation of HER2 in the human cell provides evidence of said binding. In another embodiment, the nucleic acid molecule is combined with HER3 polypeptide expressed on the surface of a human cell that further expresses HER2 polypeptide (SEQ ID NO: 6) and the method further comprises examining the human cell for evidence of

said binding, wherein the inhibition of heregulin (SEQ ID NO: 4) induced growth in the human cell provides evidence of said binding.

Another typical embodiment is a method of modulating heregulin mediated signalling in a mammalian cell, wherein the cell expresses a HER2/HER3 complex on the surface of the cell, the method comprising contacting the cell with an aptamer polynucleotide disclosed herein under conditions that allow the aptamer polynucleotide to interact with an extracellular portion of a HER3 polypeptide expressed by the cell so that heregulin mediated signalling in the mammalian cell is modulated. In a specific embodiment of the invention, the modulation of heregulin mediated signalling in a mammalian cell comprises an inhibition of heregulin mediated signalling. In an alternative embodiment, the modulation of heregulin mediated signalling in a mammalian cell comprises an enhancement of heregulin mediated signalling. In preferred embodiments, the modulation of heregulin mediated signalling in a mammalian cell comprises an inhibition of heregulin mediated signalling the mammalian cell is a human breast cancer or ovarian cancer cell.

Another typical embodiment is a method of modulating heregulin mediated signalling in a mammalian cell, wherein the cell expresses a HER2/HER3 complex on the surface of the cell, the method comprising contacting the cell with an aptamer polynucleotide disclosed herein under conditions that allow the aptamer polynucleotide to interact with an extracellular portion of a HER3 polypeptide expressed by the cell so that heregulin mediated signalling in the mammalian cell is modulated. In a specific embodiment of the invention, the modulation of heregulin mediated signalling in a mammalian cell comprises an inhibition of heregulin mediated signalling. In an alternative embodiment, the modulation of heregulin mediated signalling in a mammalian cell comprises an enhancement of heregulin mediated signalling. In preferred embodiments, the modulation of heregulin mediated signalling in a mammalian cell comprises an inhibition of heregulin mediated signalling the mammalian cell is a human breast cancer or ovarian cancer cell.

Another embodiment of the invention is a method of identifying the presence of a HER3 polypeptide in a biological sample comprising contacting the biological sample

with a aptamer disclosed herein under conditions that allow the aptamer polynucleotide to specifically bind to the HER3 polypeptide; and then examining the biological sample for the presence of a aptamer/HER3 polypeptide complex; wherein the presence of the aptamer/HER3 polypeptide complex identifies the presence of a HER3 polypeptide in the biological sample. Optionally the aptamer is labelled with a detectable label. A related embodiment of the invention is a method of identifying the presence or absence of a heregulin - HER3 polypeptide complex in a biological sample comprising contacting the biological sample with a aptamer disclosed herein under conditions that allow the aptamer polynucleotide to specifically bind to the HER3 polypeptide in the biological sample; and then examining either the affinity at which the aptamers bind the HER3 polypeptide in the biological sample; and/or the number of aptamer binding sites on the HER3 polypeptide in the biological sample; wherein the affinity at which the aptamers bind HER3 and/or the number of aptamer binding sites on HER3 is dependent upon the presence of heregulin so that the affinity at which the aptamers bind HER3 and/or the number of aptamer binding sites on HER3 identifies the presence of heregulin - HER3 polypeptide complex in a biological sample.

Another embodiment of the invention is a method of inhibiting the heregulin mediated activation of a HER2/HER3 receptor complex in a human cancer cell comprising contacting the cell with an aptamer polynucleotide comprising the sequence 5'-CAGCGAAAGUUGCGUAUGGGUCACAUCGCAG-3' (SEQ ID NO: 19) under conditions that allow the aptamer polynucleotide to interact with an extracellular portion of a HER3 polypeptide expressed by the cell such that heregulin mediated activation of a HER2/HER3 receptor is inhibited. A related embodiment of the invention is a method of inhibiting the growth of mammalian cancer cells that overexpress HER2 comprising exposing the mammalian cancer cells to a therapeutically effective amount of a composition comprising a polynucleotide having the sequence 5'-CAGCGAAAGUUGCGUAUGGGUCACAUCGCAG-3' (SEQ ID NO: 19).

Yet another embodiment of the invention is a method of enhancing the heregulin mediated activation of a HER2/HER3 receptor complex in a breast cancer cell comprising contacting the cell with an aptamer polynucleotide comprising the sequence of the A18

aptamer disclosed herein under conditions that allow the aptamer polynucleotide to interact with an extracellular portion of a HER3 polypeptide expressed by the cell such that heregulin mediated activation of a HER2/HER3 receptor is enhanced.

Another embodiment of the invention is a method for obtaining an aptamer capable of specifically binding to HER3 comprising following a SELEX methodology disclosed in Example 1B to obtain an aptamer capable of specifically adsorbing to HER3 is obtained. As the SELEX technology was used to identify aptamers from a randomized 49 nucleotide sequence, this technology is therefore also useful in the production of variants of these aptamers.

Another embodiment of the invention is a composition comprising an aptamer polynucleotide disclosed herein. In a preferred embodiment, the composition further comprises a pharmaceutically acceptable carrier. A specific embodiment of the invention is a composition comprising a polynucleotide comprising the sequence 5'-CAGCGAAAGUUGCGUAUGGGUCACAUCGCAG-3' (SEQ ID NO: 19). A related embodiment is an isolated RNA-aptamer which binds to HER3 polypeptide and competitively inhibits binding of an aptamer disclosed herein.

Also contemplated are aptamer derivatives designed to have further advantageous properties such as a high in vivo and/or serum stability. Such derivatives includes for example S-oligonucleotides (phosphorothioate derivatives or S-oligos). S-oligos (nucleoside phosphorothioates) are isoelectronic analogs of an oligonucleotide (O-oligo) in which a nonbridging oxygen atom of the phosphate group is replaced by a sulfur atom. The S-oligos may be prepared by treatment of the corresponding O-oligos with 3H-1,2-benzodithiol-3-one-1,1-dioxide, which is a sulfur transfer reagent. See Iyer, R. P. et al, 1990, J. Org. Chem. 55:4693-4698; and Iyer, R. P. et al, 1990, J. Am. Chem. Soc. 112:1253-1254, the disclosures of which are fully incorporated by reference herein. Moreover, modifications of internucleoside phosphates other than phosphorothioates may be used. Without comprising a complete listing, but as examples, one may mention methylphosphonates, phosphoroboronates, phosphoromorpholidates, butyl amidates, and peptide nucleic acid linkages. There may also be changes in the sugar ring, such as 2'-O methyl or methoxy ethoxy additions to the 2'-ribose ring. There may also be

modifications of the purine and pyrimidine bases themselves. In one illustrative embodiment, the aptamer comprises a nucleic acid sequence shown in Figure 1, wherein the nucleic acid molecule further comprises a fluorine moiety or an amino moiety.

Yet another embodiment of the invention is the use of an A30 aptamer in the manufacture of a medicament for inhibiting the heregulin-dependent tyrosine phosphorylation of HER2 in a patient. A closely related embodiment is the use of an A30 aptamer in the manufacture of a medicament for inhibiting the heregulin induced growth response of cells in a patient. In a specific embodiment of this use, the patient has a tumor comprising tumor cells expressing receptors that bind the A30 aptamer. In a specific embodiment of this use, the patient has a cancer (e.g. a breast, ovarian or colon cancer) comprising cancer cells expressing receptors that bind the A30 aptamer.

The SELEX technology disclosed herein identified the nucleic acid aptamers from a randomized polynucleotide sequence. As is known in the art, this technology can be further utilized to make and select further variants of these sequences. Consequently, the SELEX technology enables the invention disclosed herein to encompass variants of the nucleic acid sequences disclosed herein which retain their biological activities. A preferred variant is one that retains a biological activity such as the ability to bind HER3 and/or the inhibition or enhancement of heregulin mediated activities and is at least 80%, more preferably 90%, and most preferably a 95% nucleic acid sequence identity to a aptamer sequence disclosed herein (e.g. one shown in Figure 1). A related embodiment of the invention is a nucleic acid sequence which is complementary and/or hybridizes under conditions of high stringency to the nucleic acid sequences disclosed herein. The terms "complementary" or "complementarity", as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A". Complementarity between two single-stranded molecules may be "partial", in which only some of the nucleic acids bind, or it may be complete when total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency

and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands.

As used herein, the terms "hybridize", "hybridizing", "hybridizes" and the like, used in the context of polynucleotides, are meant to refer to conventional hybridization conditions, preferably such as hybridization in 50% formamide/6XSSC/0.1% SDS/100 µg/ml ssDNA, in which temperatures for hybridization are above 37 degrees C and temperatures for washing in 0.1X SSC/0.1% SDS are above 55 degrees C, and most preferably to stringent hybridization conditions. "Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature that can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., *Current Protocols in Molecular Biology*, Wiley Interscience Publishers, (1995). "Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium

chloride/sodium. citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

In the context of nucleic acid sequence comparisons, the term “identity” is used to express the percentage of nucleic acid residues at the same relative positions that are the same. Also in this context, the term “homology” is used to express the percentage of nucleic acid residues at the same relative positions that are either identical or are similar, using the conserved nucleic acid criteria of BLAST analysis, as is generally understood in the art. For example, % identity values may be generated by WU-BLAST-2 (Altschul et al., 1996, Methods in Enzymology 266:460-480; <http://blast.wustl/edu/blast/README.html>).

The invention disclosed herein further provides articles of manufacture and kits which include reagents for performing for example, the methods disclosed herein. One illustrative embodiment is a kit comprising a nucleic acid molecule comprising the sequence 5'-CAGCGAAAGUUGCGUAUGGGUCACAUCGCAG-3' (SEQ ID NO: 19) and methods for its use. Consequently, embodiment of the invention include kits and/or an article of manufacture containing materials (e.g. a HER3 aptamer polynucleotide disclosed herein) useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a HER3 aptamer composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The label on, or associated with, the container indicates that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

## EXAMPLES

### **EXAMPLE 1: ILLUSTRATIVE MATERIALS AND METHODS:**

#### **A. PRODUCTION OF HER3ECD:**

HER3ECD was produced in S2 insect cells as described previously (see, e.g. Landgraf et al. (2000) Biochemistry 39, 8503-8511). In brief, the ECDs of HER3 were cloned into the pMT/BiP/V5-His A expression vector (Invitrogen, Carlsbad, CA), which carries a metallothionine promoter and a C-terminal hexa-His and V5-epitope tag. Inductions with 500  $\mu$ M CuSO<sub>4</sub> were carried out for two days in 500 mL S2 media (Sigma, St. Louis, MO) with 10% fetal bovine serum at approximately  $6 \times 10^6$  cells/mL. The ECD was purified on a 5 mL Pharmacia HITRAP chelating column (Pharmacia, Piscataway, NJ).

#### **B. SELEX**

Single-stranded DNA templates for SELEX included 49 contiguous randomized positions flanked by constant regions (Figure 1). The constant regions included targets for PCR primers and cloning sites (*Bam*HI, *Eco*RI) as well as a T7 promoter. DNA templates (600 pmols) were transcribed *in vitro* (T7 RNA Polymerase Ribomax, Promega) and internally <sup>32</sup>P labeled RNA was purified on an 8% denaturing polyacrylamide gel. Prior to each round of selection, the RNA was denatured in phosphate-buffered saline (PBS, 150 mM NaCl, 2.5 mM KCl, 81 mM Na<sub>2</sub>HPO<sub>4</sub>, and 14.7 mM KH<sub>2</sub>PO<sub>4</sub>) at 90 °C for 10 minutes and incubated on ice for 1 min.

A filter binding assay was employed for the first eight rounds of selection. The RNA pool was first counter-selected by passing through a HAWP filter with a 0.45  $\mu$ m pore size. The counter-selected RNAs (400 pmol) were then incubated with HER3ECD at 37 °C for 10 minutes in binding buffer (10mM HEPES pH 7.4, 100mM NaCl, 2.5 mM MgCl<sub>2</sub>). Over the course of selection the ratio of protein to RNA was gradually lowered from 4:1 to 1:2. Unbound aptamers were separated from protein-bound aptamers on a HWAP filters. After two washes with PBS, bound RNA was measured by scintillation



counting of the filters and was retrieved by incubation in urea-citrate buffer (7 M urea; 0.1 M sodium citrate, pH 7.0; 3mM EDTA) at 90 °C for 10 minutes.

A gel shift assay was employed in the last seven rounds of selection. RNA (10 pmol) was incubated with HER3ECD as described above and loaded on a 6% non-denaturing acrylamide gel. Gel electrophoresis was carried out at 4 °C. The retarded band was isolated, and RNA was extracted from the gel in elution buffer (0.5 M NH<sub>4</sub>OAc, pH 7.5; 10 mM MgOAc, pH 7.0; 0.1% SDS; and 1mM EDTA) overnight at room temperature.

For both selection methods, the RNA was subsequently reverse transcribed into cDNA by avian myeloblastosis virus reverse transcriptase at 42 °C for 1 hr in a buffer purchased from Promega. Finally, the cDNA was PCR amplified for the next round of selection. Individual clones were obtained by ligation of the PCR product into either pGEM4 or pGEM3Z vectors following digestion with *Eco*RI and *Bam*HI.

#### C. GEL MOBILITY SHIFT ASSAY

For screening purposes, 8 pmol of internally labeled aptamer were incubated in binding buffer with 24 pmol of HER3ECD at 37 °C for 10 minutes and analyzed on 6% non-denaturing polyacrylamide gels as described above. Aptamers displaying substantial shift were reverse transcribed and sequenced. For subsequent analysis of A30 binding, internally labeled and unlabeled A30 was denatured at 90 °C for 2 min in PBS and cooled on ice for 1 min. A30 was incubated with HER3ECD in binding buffer for 10 min at 37 °C at the indicated concentrations and in the presence 0.5 μM tRNA and was analyzed by electrophoresis on a non-denaturing 6% polyacrylamide gel at 4 °C. Where indicated, HER3ECD and heregulin were premixed at room temperature three minutes before the addition of A30.

#### D. CELLULAR BINDING OF A30:

MCF7 cells (ATCC) were grown to 70% confluency on 6 well plates and washed with ice cold PBS. Following the washes, cells were incubated at 4°C with 1 mL ice cold

PBS or PBS containing 200 nM heregulin. After four hours an equal volume of ice cold PBS containing two microliter internally  $^{32}\text{P}$  labeled A30 aptamer (80,000 cpm/ $\mu\text{L}$ ), 20  $\mu\text{L}$  RNase inhibitor (20 units/ $\mu\text{L}$ ), and varying concentrations of unlabeled aptamer were added. After four hours incubation, the supernatant was removed, and cells were washed three times with ice cold PBS. Displaced and bound radiolabeled aptamer was measured in duplicate.

**E. Tyrosine phosphorylation assay:**

MCF7 cells were seeded two days prior to the experiment in RPMI with 10% fetal bovine serum. Cells were serum starved 24 hours prior to stimulation. Following two washes with PBS, cells were stimulated with heregulin or heregulin plus different concentrations of aptamer in RPMI. After 15 minutes of stimulation at 37 °C, cells were placed on ice and washed twice with ice cold PBS. Cells were lysed with mild lysis buffer (20 mM Tris 8.0, 137 mM NaCl, 1% Triton X-100, 10% glycerol, 5 mM EDTA, 1 mM sodium orthovanadate, 1 mM phenylmethyl-sulfinylfluoride, 1 $\mu\text{g}/\text{mL}$  leupeptin, 1 $\mu\text{g}/\text{ml}$  aprotinin). Lysates were either evaluated directly for tyrosine phosphorylation or were first subjected to immunoprecipitation in mild lysis buffer as described previously (see, e.g. Landgraf et al. (2000) Biochemistry 39, 8503-8511) using anti-HER2 antibody (Ab3, Oncogene) and ProteinA/G beads (Santa Cruz Biotech). Western blot analysis was done using an anti-phosphotyrosine antibody (4G10, Upstate) as the primary, and anti-mouse IgG-HRP conjugates (Upstate) as secondary antibody.

**F. CELL PROLIFERATION ASSAY:**

MCF7 cells were seeded in 96 well plates (2500 cells/well) in RPMI, 2% fetal bovine serum, 2 units of RNase inhibitor/ml and varying concentrations of heregulin and aptamer. After two days, cell growth was determined using an MTT/tetrazolium based assay (Promega). Cell growth was equated with the absorbance of converted and solubilized dye at 560 nm. All samples were determined in triplicate.

**EXAMPLE 2: SELECTION OF RNA APTAMERS THAT BIND HER3ECD.**

HER3ECD has a molecular weight of 82 kD, which includes 12% carbohydrates (see, e.g. Landgraf et al. (2000) *Biochemistry* 39, 8503-8511), and represents an exceptionally large target for SELEX. At the high concentrations of HER3ECD required for SELEX, the ECD is completely in its oligomeric state. Previous analysis suggests that the upper limit of self-association in solution are twelve copies of the ECD (see, e.g. Landgraf et al. (2000) *Biochemistry* 39, 8503-8511).

The analysis of 88 clones, obtained after 15 rounds of SELEX, identified 29 clones that gave reproducible positive results in gel mobility shift assays with HER3ECD. All 29 clones were sequenced. The sequences of six aptamers with good gel-shift properties are disclosed herein. Beyond an apparent bias for adenine in the first half and uracil in the second half of the aptamers, we could not identify a consensus pattern. This apparent lack of a consensus among the 29 sequences could indicate insufficient sample sequences, several distinct bindings sites on the target, or both. Given the exceptionally large size of the ECD, the possibility of multiple target sites is plausible.

Depending on their site of binding, aptamers could interfere with receptor self-association, heterodimerization or ligand binding. We evaluated different aptamers for their ability to interfere with heregulin-induced tyrosine phosphorylation of HER2. While "low-affinity" binding of heregulin to HER3 has a  $K_d$  of 2-8 nM, "high-affinity" binding to the HER3-HER2 heterodimer has a  $K_d$  of  $\sim 10^{-10}$  M (see, e.g. Tzahar et al. (1994) *J Biol Chem* 269, 25226-33) and results primarily in the phosphorylation of HER2. For the initial screen, we used an  $\Omega$ -loop mutant of heregulin with reduced binding affinity. The  $\Omega$ -loop is not essential for activity but multiple alanine mutations in this loop reduce the binding affinity towards HER3 (see, e.g. Jones et al. (1998) *J Biol Chem* 273, 11667-74). Figure 2 shows the results obtained for the six aptamers shown in Figure 1. Although all 29 of 88 aptamers were selected based on their ability to bind HER3ECD, they differ in their effect on receptor stimulation. Aptamer 19 shows little interference with heregulin-dependent activation while aptamer 30, and to a lesser extent 23 and 37, show inhibition. In contrast, aptamer 6 and 18 enhance heregulin dependent

activation. A18 reproducibly caused a 24 ( $\pm 4$ )% enhancement of heregulin stimulation in independent experiments. Further comparison of aptamer 18 and 30 revealed that neither elicits tyrosine phosphorylation by itself or displaces the other aptamer from its binding site on HER3ECD. Although the aptamers shown in Figure 1 and 2 represent only a subset of all obtained sequences, the apparent qualitative differences in their activities and the lack of mutual competition of A18 and A30 are consistent with the assumption of at least two distinct binding sites. Because of the potency of A30 and the possibilities inherent in an aptamer with inhibitory properties, we focused on A30 for the remainder of this study.

### EXAMPLE 3: SPECIFICITY OF A30:

Given the high homology between HER3 and HER2, we next confirmed the specificity of A30 binding (Figure 3). A gel-shift of A30 was observed only for HER3ECD (lane 3), but not for HER2-ECD (lane 2) or heregulin (lane 1). Furthermore, binding of A30 to HER3ECD cannot be inhibited by a 20-fold molar excess of tRNA (lane 4). Combined with the fact that the majority of aptamer sequences does not bind HER3ECD at all, these results indicate a high level of specificity in the interaction of HER3ECD and A30.

### EXAMPLE 4: MODE OF INTERACTION BETWEEN A30 AND HER3ECD:

Several lines of evidence provide evidence that the interaction of A30 with HER3ECD involves at least two different modes of binding. Figure 4A shows A30 binding to the ECD in the presence and absence of heregulin, as determined by a gel shift assay. In the absence of heregulin, an apparent  $K_d$  of 45 nM is obtained. Addition of heregulin has two consequences. The number of binding sites increases nine-fold, and the binding affinity decreases to an apparent  $K_d$  of 400 nM. With respect to the heregulin-induced increase and dual mode of binding, we observed a similar, although less pronounced, effect for A30 binding to MCF7 cells (Figure 4B+C). MCF7 cells endogenously express both HER3 and HER2. Overall, binding to MCF7 cells at 4 °C is tighter with a  $K_d$  of 21 nM ( $\pm 2.2$ ) and 3.3 nM ( $\pm 0.2$ ) respectively for low and high

affinity binding (Figure 4C). The addition of heregulin results in an increase in the number of A30 binding sites (Figure 4B), but this increase is relatively small (25% total, 17% for low affinity and 8 % for high affinity sites).

Across experiments, gelshifts of A30 with HER3ECD produce a set of three bands (A, B, C in Figure 3 and 5), the ratio of which can differ based on the concentration of components as well as the particular batches of refolded A30. A direct comparison of binding with the same batch of A30 in the presence and absence of heregulin shows that bands that are not derived from A30 alone (Ctrl, lane 1 Figure 5) are qualitatively the same in the presence and absence of heregulin (which contributes minimal changes in both the size and charges of the complex compared to A30). When the concentration of unlabeled A30 is increased, the faster migrating species (B and C) are more readily subject to competition. Upon addition of heregulin, increases in binding are primarily observed for the faster migrating species B and C.

We previously showed that HER3ECD has a high propensity to form oligomers in solution. Those oligomers dissociate in the presence of excess heregulin. To evaluate the binding preferences of A30 for the oligomeric versus the monomeric state of HER3ECD, we visualized HER3ECD as well as radiolabeled A30. Because of the close to neutral charges of the HER3ECD and HER3ECD-heregulin complex, both species are not well separated under gelshift conditions that are optimized for the highly charged and the fast migrating complexes with A30. A significant shift can be observed upon heregulin binding to HER3ECD in Phast gels®, probably as a result of the significantly higher current flow allowable in these systems. However, the nature of these gels makes Western blot analysis difficult. We therefore decided to visualize purified HER3ECD directly at high protein concentrations.

Figure 6 shows the obtained shifts, visualized either through autoradiography of radiolabeled A30 or by coomassie staining. Radiolabeled A30 (0.3 nM) is present in all lanes. Lanes 3 and 4 show the partial gelshift of 2  $\mu$ M HER3ECD with a 3 fold molar excess of heregulin, visualized by coomassie staining. Radiolabeled A30 binds to the oligomeric species of HER3ECD (lane 4 and 9). Under conditions when HER3ECD oligomers are in excess over A30 and disruption of HER3ECD oligomers by heregulin is

only partial (lane 3 and 8). The simultaneous presence of oligomeric and monomeric species of HER3, detectable by coomassie staining, allows a direct comparison of A30 binding to both species. A30 preferentially binds the oligomeric species of HER3ECD. A small amount of an additional faster migrating species is visible on the autoradiography in both cases. Using a four-fold molar excess of A30 over HER3ECD results in three shifted species of HER3ECD, visible by direct coomassie staining (Figure 6, lane 2). This comparison identifies the additional band (species c) in lanes 8 and 9 as a small portion of A30-shifted HER3ECD. The addition of heregulin does not alter this pattern. The assignment of species a, b and c in this experiment is based on the similarity in the pattern of bands with the gelshifts in Figure 3 and 5 (lane 5). The distinction of lower and upper case letters reflects the fact that the equivalence of those bands has not been confirmed in light of the differences in the two gel systems.

#### **EXAMPLE 5: INHIBITION OF TYROSINE PHOSPHORYLATION AND GROWTH STIMULATION**

Next, we evaluated the ability of A30 to interfere with receptor activation by wild type heregulin. Figure 7 shows HER2 tyrosine phosphorylation in MCF7 cells following stimulation by heregulin. Half-maximal inhibition of tyrosine phosphorylation occurs at a concentration around 10 nM A30 (Fig 7a). In contrast, the activation of HER2 by EGF, which proceeds through heterodimers of EGFR and HER2, is not inhibited by A30 (Fig. 7b). The low intensity of tyrosine phosphorylation of HER2 after stimulation by EGF is a reflection of the lower levels of EGFR (5000/cell) compared to HER2 (15,000) and HER3 (25,000) (see, e.g. Aguilar et al. (1999) *Oncogene* 18, 6050-62) and the fact that EGFR signaling proceeds only in part through heterodimers with HER2. To evaluate if the inhibition of tyrosine phosphorylation is reflected in a reduction in heregulin-specific growth stimulation, we incubated MCF7 cells in the presence of different concentrations of A30 and heregulin for two days (Figure 8). The addition of A30 results in a 50% inhibition of heregulin-specific growth stimulation, even at high concentrations (100nM) of heregulin. Half-maximal inhibition occurs at around 1 nM A30. The addition of A30 alone has little effect on the growth of MCF7 cells.

\* \* \* \* \*

Throughout this application, various publications are referenced (e.g. Chen et al., Proc Natl Acad Sci U S A. 2003;100(16): 9226-31). The disclosures of these publications are hereby incorporated by reference herein in their entireties. The technology in this application is also related to U.S. Application Serial No. 10/207,498 filed July 29, 2002, the contents of which are incorporated herein by reference.

The present invention is not to be limited in scope by the embodiments disclosed herein, which are intended as single illustrations of individual aspects of the invention, and any that are functionally equivalent are within the scope of the invention. Various modifications to the models and methods of the invention, in addition to those described herein, will become apparent to those skilled in the art from the foregoing description and teachings, and are similarly intended to fall within the scope of the invention. Such modifications or other embodiments can be practiced without departing from the true scope and spirit of the invention.

**Table 1:** Illustrative HER3, Heregulin and HER2 polynucleotide and polypeptide sequences:

**1A. HER3 Polynucleotide Sequence**

ATGAGGGCGAACGACGCTCTGCAGGTGCTGGGCTTGCTTTTCAGCCTGGCCCGGGGC  
TCCGAGGTGGGCAACTCTCAGGCAGTGTGTCCTGGGACTCTGAATGGCCTGAGTGTG  
ACCGGCGATGCTGAGAACCAATACCAGACACTGTACAAGCTCTACGAGAGGTGTGAG  
GTGGTGATGGGGAACCTTGAGATTGTGCTCACGGGACACAATGCCGACCTCTCCTTC  
CTGCAGTGGATTGAGAAGTGACAGGCTATGTCCTCGTGGCCATGAATGAATTCTCT  
ACTCTACCATTGCCCAACCTCCGCGTGGTGCGAGGGACCCAGGTCTACGATGGGAAG  
TTTGCCATCTTCGTCATGTTGAACTATAACCAACTCCAGCCACGCTCTGCGCCAG  
CTCCGCTTGACTCAGCTCACCGAGATTCTGTCAGGGGGTGTTTATATTGAGAAGAAC  
GATAAGCTTTGTACATGGACACAATTGACTGGAGGGACATCGTGAGGGACCGAGAT  
GCTGAGATAGTGGTGAAGGACAATGGCAGAAGCTGTCCCCCTGTCATGAGGTTTGC  
AAGGGGCGATGCTGGGGTCTGGATCAGAAGACTGCCAGACATTGACCAAGACCATC  
TGTGCTCCTCAGTGTAATGGTCACTGCTTTGGGCCCCAACCCCAACCAGTGCTGCCAT  
GATGAGTGTGCCGGGGGCTGCTCAGGCCCTCAGGACACAGACTGCTTTGCCTGCCGG  
CACTTCAATGACAGTGGAGCCTGTGTACCTCGCTGTCCACAGCCTCTTGTCTACAAC

AAGCTAACTTTCCAGCTGGAACCCAATCCCCACACCAAGTATCAGTATGGAGGAGTT  
TGTGTAGCCAGCTGTCCCCATAACTTTGTGGTGGATCAAACATCCTGTGTCAGGGCC  
TGTCTCCTGACAAGATGGAAGTAGATAAAAAATGGGCTCAAGATGTGTGAGCCTTGT  
GGGGGACTATGTCCCAAAGCCTGTGAGGGAACAGGCTCTGGGAGCCGCTTCCAGACT  
GTGGACTCGAGCAACATTGATGGATTTGTGAACTGCACCAAGATCCTGGGCAACCTG  
GACTTTCTGATCACCGGCCTCAATGGAGACCCCTGGCACAAGATCCCTGCCCTGGAC  
CCAGAGAAGCTCAATGTCTTCCGGACAGTACGGGAGATCACAGGTTACCTGAACATC  
CAGTCCTGGCCGCCCCACATGCACAACTTCAGTGTTTTTTCCAATTTGACAACCATT  
GGAGGCAGAAGCCTCTACAACCGGGGCTTCTCATTGTTGATCATGAAGAACTTGAAT  
GTCACATCTCTGGGCTTCCGATCCCTGAAGGAAATTAGTGCTGGGCGTATCTATATA  
AGTGCCAATAGGCAGCTCTGCTACCACCACTCTTTGAACTGGACCAAGGTGCTTCGG  
GGGCTACGGAAGAGCGACTAGACATCAAGCATAATCGGCCGCGCAGAGACTGCGTG  
GCAGAGGGCAAAGTGTGTGACCCACTGTGCTCCTCTGGGGGATGCTGGGGCCCAGGC  
CCTGGTCAGTGCTTGTCTGTGCGAAATTATAGCCGAGGAGGTGTCTGTGTGACCCAC  
TGCAACTTTCTGAATGGGGAGCCTCGAGAATTTGCCCATGAGGCCGAATGCTTCTCC  
TGCCACCCGGAATGCCAACCCATGGGGGGCACTGCCACATGCAATGGCTCGGGCTCT  
GATACTTGTGCTCAATGTGCCCATTTCGAGATGGGCCCCACTGTGTGAGCAGCTGC  
CCCCATGGAGTCCTAGGTGCCAAGGGCCCAATCTACAAGTACCCAGATGTTTCAAGAT  
GAATGTGCGGCCCTGCCATGAGAAGTGCACCCAGGGGTGTAAAGGACCAGAGCTTCAA  
GACTGTTTATAGACAAACACTGGTGCTGATCGGCAAAACCCATCTGACAATGGCTTTG  
ACAGTGATAGCAGGATTGGTAGTGATTTTCATGATGCTGGGCGGCACTTTTCTCTAC  
TGGCGTGGGCGCCGGATTGAGAATAAAAGGGCTATGAGGCGATACTTGGAACGGGGT  
GAGAGCATAGAGCCTCTGGACCCCACTGAGAAGGCTAACAAAGTCTTGGCCAGAATC  
TTCAAAGAGACAGAGCTAAGGAAGCTTAAAGTGCTTGCGTCTGGGTGTCTTTGGAAGT  
GTGCACAAAGGAGTGTGGATCCCTGAGGGTGAATCAATCAAGATTCCAGTCTGCATT  
AAAGTCATTGAGGACAAGAGTGGACGGCAGAGTTTTCAGCTGTGACAGATCATATG  
CTGGCCATTGGCAGCCTGGACCATGCCACATTGTAAGGCTGCTGGGACTATGCCCA  
GGGTCACTCTCTGCAGCTTGTCACTCAATATTTGCCTCTGGGTTCTCTGCTGGATCAT  
GTGAGACAACACCGGGGGGCACTGGGGCCACAGCTGCTGCTCAACTGGGGAGTACAA  
ATTGCCAAGGGAATGTACTACCTTGAGGAACATGGTATGGTGCATAGAAACCTGGCT  
GCCCCAAACGTGCTACTCAAGTCACCCAGTCAGGTTTCAAGTGGCAGATTTTGGTGTG  
GCTGACCTGCTGCCTCCTGATGATAAGCAGCTGCTATACAGTGAGGCCAAGACTCCA  
ATTAAGTGGATGGCCCTTGAGAGTATCCACTTTGGGAAATACACACACCAGAGTGAT  
GTCTGGAGCTATGGTGTGACAGTTTGGGAGTTGATGACCTTCGGGGCAGAGCCCTAT  
GCAGGGCTACGATTGGCTGAAGTACCAGACCTGCTAGAGAAGGGGGAGCGGTTGGCA  
CAGCCCCAGATCTGCACAATTGATGTCTACATGGTGTGATGGTCAAGTGTGATGATT  
GATGAGAACATTGCCCCAACCTTTAAAGAACTAGCCAATGAGTTTACCAGGATGGCC  
CGAGACCCACCACGGTATCTGGTCATAAAGAGAGAGAGTGGGCCTGGAATAGCCCCT  
GGGCCAGAGCCCCATGGTCTGACAAACAAGAAGCTAGAGGAAGTAGAGCTGGAGCCA  
GAACTAGACCTAGACCTAGACTTGAAGCAGAGGAGGACAACCTGGCAACCACCACA  
CTGGGCTCCGCCCTCAGCCTACCAGTTGGAACACTTAATCGGCCACGTGGGAGCCAG  
AGCCTTTTAAAGTCCATCATCTGGATACATGCCCATGAACCAGGGTAATCTTGGGGGG  
TCTTGCCAGGAGTCTGCAGTTTCTGGGAGCAGTGAACGGTGCCCCCGTCCAGTCTCT  
CTACACCCAATGCCACGGGGATGCCTGGCATCAGAGTCATCAGAGGGGCATGTAACA



GGCTCTGAGGCTGAGCTCCAGGAGAAAGTGTCAATGTGTAGAAAGCCGGAGCAGGAGC  
CGGAGCCCACGGCCACGCGGAGATAGCGCCTACCATTCCCAGCGCCACAGTCTGCTG  
ACTCCTGTTACCCCACTCTCCCCACCCGGGTTAGAGGAAGAGGATGTCAACGGTTAT  
GTCATGCCAGATACACACCTCAAAGGTACTCCCTCCTCCCGGAAGGCACCCTTTCT  
TCAGTGGGTCTCAGTTCTGTCTGCTGGGTACTGAAGAAGAAGATGAAGATGAGGAGTAT  
GAATACATGAACCGGAGGAGAAGGCACAGTCCACCTCATCCCCCTAGGCCAAGTTCC  
CTTGAGGAGCTGGGTTATGAGTACATGGATGTGGGGTCAGACCTCAGTGCCTCTCTG  
GGCAGCACACAGAGTTGCCCACTCCACCCTGTACCCATCATGCCCACTGCAGGCACA  
ACTCCAGATGAAGACTATGAATATATGAATCGGCAACGAGATGGAGGTGGTCTCTGGG  
GGTGATTATGCAGCCATGGGGGCTGCCCAGCATCTGAGCAAGGGTATGAAGAGATG  
AGAGCTTTTCAGGGGCTGGACATCAGGCCCCCATGTCCATTATGCCCGCCTAAAA  
ACTCTACGTAGCTTAGAGGCTACAGACTCTGCCTTTGATAACCCTGATTACTGGCAT  
AGCAGGCTTTTCCCAAGGCTAATGCCCAGAGAACG SEQ ID NO: 1

1B. HER3 Polypeptide Sequence

MRANDALQVLGLLFSLARGSEVGN SQAVCPGTLNGLSVTGDAENQYQTLYKLYERCE  
VVMGNLEIVLTGHNADLSFLQWIREVTGYVLVAMNEFSTLPLPNLRVVRGTQVYDGK  
FAIFVMLNNTNSSHALRQLRLTQLTEILSGGVYIEKNDKLCHMDTIDWRDIVRDRD  
AEIVVKDNGRSCPPCHEVCKGRCWGPGEQTLTKTICAPQCNGHCFGNPNQCCH  
DECAGGCSGPQDTCFACRHFNDGACVPRCPQPLVYNKLTQLEPNPHTKYQYGGV  
CVASCPHNFVVDQTSVVRACPPDKMEVDKNGLKMCEPCGGLCPKACEGTGSGSRFQT  
VDSSNIDGFVNCTKILGNLDFLITGLNGDPWHKIPALDPEKLNVFRTVREITGYLNI  
QSWPPHMHNFVSFNLTTIGGRSLYNRGFSLIMKNLNVTSLGFRSLKEISAGRIYI  
SANRQLCYHHSNLNWKVLRGPTEERLDIKHNRPRRDCVAEGKVCDPLCSSGGCWGPG  
PGQCLSCRNYSRGGVCVTHCNFLNGEPREFAEAECSCHPECQPMGGTATCNGSGS  
DTCAQCAHFRDGPHCVSSCPHGVLGAKGPIYKYPDVQNECRPCHENCTQGCKGPQLQ  
DCLGQTLVLIGKTHLTMALTVIAGLVVIFMMLGGTFLYWRGRRIQNKRMRRYLERG  
ESIEPLDPSEKANKVLARIFKETELRKLKVLGSGVFGTVHKGWVWIPAGESIKIPVCI  
KVIEDKSGRQSFQAVTDHMLAIGSLDHAHIVRLLGLCPGSSLQLVTOYLPLGSLLDH  
VRQHRGALGPQLLLNWGVQIAKGMYYLEEHGMVHRNLAARNVLLKSPSQVQVADFGV  
ADLLPPDDKQLLYSEAKTPIKWMALSIHFGKYTHQSDVWSYGVTVWELMTFGAEPY  
AGLRLAEVPDLLEKGERLAQPQICTIDVYVMVMVKCWMIDENIRPTFKELANEFTRMA  
RDPPLYLVIKRESGPGIAPGPEPHGLTNKKLEEVELEPELDLDLDLEAEEDNLATTT  
LGSALSPLVGTNLNRPRGSQSLLSPSSGYMPMNQGNLGGSCQESAVSGSSERCPRPVS  
LHPMPRGCLASESSEGHVTGSEAELOEKVSMCRSRSRSPRPRGDSAYHSQRHSL  
TPVTPLSPGLEEEDVNGYVMPDTHLKGTPSSREGTLSSVGLSSVLGTEEEDEDEEY  
EYMNRRRRHSPHPHPPRPSLEELGYEYMDVGSLSASLGSTQSCPLHPVPIMPTAGT  
TPDEDYEYMNQRDGGGPGGDYAAMGACPAEQGYEEMRAFGQPGHQAPHVHYARLK  
TLRSLEATDSAFDNPDYWHSRLFPKANAQRT SEQ ID NO: 2

## 1C. Heregulin Polynucleotide Sequence

ATGTCCGAGCGCAAAGAAGGCAGAGGCCAAAGGGAAGGGCAAGAAGAAGGAGCGAGGC  
TCCGGCAAGAAGCCGGAGTCCGCGGCGGGCAGCCAGAGCCCAGCCTTGCCCTCCCCAA  
TTGAAAGAGATGAAAAGCCAGGAATCGGCTGCAGGTTCCAAACTAGTCCTTCGGTGT  
GAAACCAGTTCTGAATACTCCTCTCTCAGATTCAAGTG GTTCAAGAATGGGAATGAA  
TTGAATCGAAAAACAAACCACAAATATCAAGATACAAAAAAGCCAGGGAAGTCA  
GAACTTCGCATTAACAAAGCATCACTGGCTGATTCTGGAGAGTATATGTGCAAAGTG  
ATCAGCAAATTAGGAAATGACAGTGCCCTCTGCCAATATCACCATCGTGGAATCAAAC  
GAGATCATCACTGGTATGCCAGCCTCAACTGAAGGAGCATATGTGTCTTCAGAGTCT  
CCCATTAGAATATCAGTATCCACAGAAGGAGCAAATACTTCTTCATCTACATCTACA  
TCCACCACTGGGACAAGCCATCTTGTAATATGTGCGGAGAAGGAGAAAAC TTTCTGT  
GTGAATGGAGGGGAGTGCTTCATGGTGAAAGACCTTTCAAACCCCTCGAGATACTTG  
TGCAAGTGCCCAAATGAGTTTACTGGTGATCGCTGCCAAAAC TACGTAATGGCCAGC  
TTCTACAAGCATCTTGGGATTGAATTTATGGAGGCGGAGGAGCTGTACCAGAAGAGA  
GTGCTGACCATAACCGGCATCTGCATCGCCCTCCTTGTGGTCGGCATCATGTGTGTG  
GTGGCCTACTGCAAAACCAAGAAACAGCGGAAAAAGCTGCATGACCGTCTTCGGCAG  
AGCCTTCGGTCTGAACGAAACAATATGATGAACATTGCCAATGGGCCTCACCATCCT  
AACCCACCCCCCGAGAATGTCCAGCTGGTGAATCAATACGTATCTAAAAACGTCATC  
TCCAGTGAGCATATTGTTGAGAGAGAAGCAGAGACATCCTTTTCCACCAGTCACTAT  
ACTTCCACAGCCCATCACTCCACTACTGTCACCAGACTCCTAGCCACAGCTGGAGC  
AACGGACACACTGAAAGCATCCTTTCCGAAAGCCACTCTGTAATCGTGATGTCATCC  
GTAGAAAACAGTAGGCACAGCAGCCCAACTGGGGGCCCAAGAGGACGTCTTAATGGC  
ACAGGAGGCCCTCGTGAAATGTAACAGCTTCCTCAGGCATGCCAGAGAAACCCCTGAT  
TCCTACCGAGACTCTCCTCATAGTGAAAGGTATGTGTCAGCCATGACCACCCCGGCT  
CGTATGTCACCTGTAGATTTCCACACGCCAAGCTCCCCCAAATCGCCCCCTTCGGAA  
ATGTCTCCACCCGTGTCCAGCATGACGGTGTCCATGCCTTCCATGGCGGTGAGCCCC  
TTCATGGAAGAAGAGAGACCTCTACTTCTCGTGACACCACCAAGGCTGCGGGAGAAG  
AAGTTTGACCATCACCTCAGCAGTTCAGCTCCTTCCACCACAACCCCGCGCATGAC  
AGTAACAGCCTCCCTGCTAGCCCCTTGAGGATAGTGAGGATGAGGAGTATGAAACG  
ACCCAAGAGTACGAGCCAGCCCAAGAGCCTGTTAAGAAACTCGCCAATAGCCGGCGG  
GCCAAAAGAACCAAGCCCAATGGCCACATTGCTAACAGATTGGAAGTGGACAGCAAC  
ACAAGCTCCAGAGCAGTAACTCAGAGAGTGAAACAGAAGATGAAAGAGTAGGTGAA  
GATACGCCTTTCTTGGGCATACAGAACCCCTGGCAGCCAGTCTTGAGGCAACACCT  
GCCTTCCGCCTGGCTGACAGCAGGACTAACCCAGCAGGCCGCTTCTCGACACAGGAA  
GAAATCCAGGCCAGGCTGTCTAGTGTAATTGCTAACCAAGACCCTATTGCTGTA  
SEQ ID NO: 3

## 1D. Heregulin Polypeptide Sequence

MSEKKEGRGKGKGGKKKERGSGKKPESAAGSQSPALPPRLKEMKSQESAAGSKLVLRCE  
ETSSEYSSSLRFKWFKNGNELNRKNKPQNIKIQKKPGKSELRINKASLADSGEYMCKV  
ISKLGNDASANITIVESNEIITGMPASTEGAYVSSESPIRISVSTEGANTSSSTST  
STTGTSHLVKCAEKEKTFVCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMAS  
FYKHLGIEFMEAEELYQKRVLTITGICIALLVGIMCVVAYCKTKKQRKKLHDLRQ  
SLRSENNMMNIANGPHHPNPPPENVLVNQYVSKNVISSEHIVEREAETSFSSTSHY  
TSTAHHSTTVTQTPSHSWSNGHTESILSESHSVIVMSSVENSRRHSSPTGGPRGRLNG  
TGGPRECNSFLRHARETPDSYRDSPHSERYVSAMTTPARMSPVDFHTPSSPKSPSE  
MSPPVSSMTVSMPSMAVSPFMEEERPLLLVTPPRLREKKFDHHPQQFSSFHHNPAHD  
SNSLPASPLRIVEDEEYETTQEYEPAQEPVKKLANSRRAKRTKPNGHIANRLEVDSN  
TSSQSSNSESETEDERVGEDTPFLGIQNPLAASLEATPAFRLADSRTPAGRFSTQE  
EIQARLSSVIANQDPIAV SEQ ID NO: 4

## 1E. HER2 Polynucleotide Sequence

ATGGAGCTGGCGGCCTTGTGCCGCTGGGGGCTCCTCCTCGCCCTCTTGCCCCCGGA  
GCCGCGAGCACCCAAGTGTGCACCGGCACAGACATGAAGCTGCGGCTCCCTGCCAGT  
CCCGAGACCCACCTGGACATGCTCCGCCACCTCTACCAGGGCTGCCAGGTGGTGACAG  
GGAAACCTGGAACTCACCTACCTGCCCACCAATGCCAGCCTGTCCTTCTGCAGGAT  
ATCCAGGAGGTGCAGGGCTACGTGCTCATCGCTCACAACCAAGTGAGGCAGGTCCCA  
CTGCAGAGGCTGCGGATTGTGCGAGGCACCCAGCTCTTTGAGGACAACCTATGCCCTG  
GCCGTGCTAGACAATGGAGACCCGCTGAACAATACCACCCCTGTACAGGGGCTCC  
CCAGGAGGCCTGCGGGAGCTGCAGCTTCTGAAGCCTCACAGAGATCTTGAAAGGAGGG  
GTCTTGATCCAGCGGAACCCCCAGCTCTGCTACCAGGACACGATTTTGTGGAAGGAC  
ATCTTCCACAAGAACAACCAAGCTGGCTCTCACACTGATAGACACCAACCGCTCTCGG  
GCCTGCCACCCCTGTTCTCCGATGTGTAAGGGCTCCCGCTGCTGGGGAGAGAGTTCT  
GAGGATTGTGAGAGCCTGACGCGCACTGTCTGTGCCGGTGGCTGTGCCCGCTGCAAG  
GGGCCACTGCCCACTGACTGCTGCCATGAGCAGTGTGCTGCCGGCTGCACGGGCCCC  
AAGCACTCTGACTGCCTGGCCTGCCTCCACTTCAACCACAGTGGCATCTGTGAGCTG  
CACTGCCCAGCCCTGGTACCTACAACACAGACACGTTTGAGTCCATGCCCAATCCC  
GAGGGCCCGGTATACATTCCGGCGCCAGCTGTGTGACTGCCTGTCCCTACAACCTACCTT  
TCTACGGACGTGGGATCCTGCACCCCTCGTCTGCCCCCTGCACAACCAAGAGGTGACA  
GCAGAGGATGGAACACAGCGGTGTGAGAAGTGCAGCAAGCCCTGTGCCCGAGTGTGC  
TATGGTCTGGGCATGGAGCACTTGCAGAGAGGTGAGGGCAGTTACCAGTGCCAATATC  
CAGGAGTTTGTGCTGGCTGCAAGAAGATCTTTGGGAGCCTGGCATTCTGCCGGAGAGC  
TTTGATGGGGACCCAGCCTCCAACACTGCCCCGCTCCAGCCAGAGCAGCTCCAAGTG  
TTTGAGACTCTGGAAGAGATCACAGGTTACCTATACATCTCAGCATGGCCGGACAGC  
CTGCCTGACCTCAGCGTCTTCCAGAACCTGCAAGTAATCCGGGGACGAATTCTGCAC  
AATGGCGCCTACTCGCTGACCTTGCAAGGGCTGGGCATCAGCTGGCTGGGGCTGCGC  
TCACTGAGGGAACCTGGGCAGTGGACTGGCCCTCATCCACCATAACACCCACCTCTGC  
TTCGTGCACACGGTGCCCTGGGACCAGCTCTTTCGGAACCCGCACCAAGCTCTGCTC  
CACACTGCCAACC GGCCAGAGGACGAGTGTGTGGGCGAGGGCCTGGCCTGCCACCAG

CTGTGCGCCCGAGGGCACTGCTGGGGTCCAGGGCCACCCAGTGTGTCAACTGCAGC  
CAGTTCCTTCGGGGCCAGGAGTGCGTGAGGAATGCCGAGTACTGCAGGGGCTCCCC  
AGGGAGTATGTGAATGCCAGGCACTGTTTGCCGTGCCACCCTGAGTGTGAGCCCCAG  
AATGGCTCAGTGACCTGTTTTGGACCGGAGGCTGACCAGTGTGTGGCCTGTGCCCCAC  
TATAAGGACCCTCCCTTCTGCGTGCGCCGCTGCCCCAGCGGTGTGAAACCTGACCTC  
TCCTACATGCCCATCTGGAAGTTTCCAGATGAGGAGGGCGCATGCCAGCCTTGCCCC  
ATCAACTGCACCCACTCCTGTGTGGACCTGGATGACAAGGGCTGCCCCGCCGAGCAG  
AGAGCCAGCCCTCTGACGTCCATCGTCTCTGCGGTGGTTGGCATTCTGCTGGTCTGTG  
GTCTTGGGGGTGGTCTTTGGGATCCTCATCAAGCGACGGCAGCAGAAGATCCGGAAG  
TACACGATGCGGAGACTGCTGCAGGAAACGGAGCTGGTGGAGCCGCTGACACCTAGC  
GGAGCGATGCCCAACCAGGCGCAGATGCGGATCCTGAAAGAGACGGAGCTGAGGAAG  
GTGAAGGTGCTTGGATCTGGCGCTTTTGGCACAGTCTACAAGGGCATCTGGATCCCT  
GATGGGGAGAATGTGAAAATTCAGTGGCCATCAAAGTGTGAGGGAAAACACATCC  
CCCAAAGCCAACAAGAAATCTTAGACGAAGCATACTGATGGCTGGTGTGGGCTCC  
CCATATGTCTCCCGCCTTCTGGGCATCTGCCTGACATCCACGGTGACGCTGGTGACA  
CAGCTTATGCCCTATGGCTGCCTCTTAGACCATGTCCGGGAAAACCGCGGACGCCTG  
GGCTCCAGGACCTGCTGAACTGGTGTATGCAGATTGCCAAGGGGATGAGCTACCTG  
GAGGATGTGCGGCTCGTACACAGGGACTTGGCCGCTCGGAACGTGCTGGTCAAGAGT  
CCCAACCATGTCAAAATTACAGACTTCGGGCTGGCTCGGCTGCTGGACATTGACGAG  
ACAGAGTACCATGCAGATGGGGGCAAGGTGCCCATCAAGTGGATGGCGCTGGAGTCC  
ATTCTCCGCGGCGGTTTACCCACCAGAGTGATGTGTGGAGTTATGGTGTGACTGTG  
TGGGAGCTGATGACTTTTGGGGCCAAACCTTACGATGGGATCCCAGCCCCGGGAGATC  
CCTGACCTGCTGGAAAAGGGGGAGCGGCTGCCCCAGCCCCCATCTGCACCATTGAT  
GTCTACATGATCATGGTCAAATGTTGGATGATTGACTCTGAATGTGCGCCAAGATTC  
CGGGAGTTGGTGTCTGAATTCTCCCGCATGGCCAGGGACCCCCAGCGCTTTGTGGTC  
ATCCAGAATGAGGACTTGGGCCCAGCCAGTCCCTTGGACAGCACCTTCTACCGCTCA  
CTGCTGGAGGACGATGACATGGGGGACCTGGTGGATGCTGAGGAGTATCTGGTACCC  
CAGCAGGGCTTCTTCTGTCCAGACCCTGCCCCGGGCGCTGGGGGCATGGTCCACCAC  
AGGCACCGCAGCTCATCTACCAGGAGTGGCGGTGGGGACCTGACACTAGGGCTGGAG  
CCCTCTGAAGAGGAGGCCCCCAGGTCTCCACTGGCACCTCCGAAGGGGCTGGCTCC  
GATGTATTTGATGGTGACCTGGGAATGGGGGCAGCCAAGGGGCTGCAAAGCCTCCCC  
ACACATGACCCCAGCCCTCTACAGCGGTACAGTGAGGACCCCAAGTACCCCTGCCC  
TCTGAGACTGATGGCTACGTTGCCCCCTGACCTGCAGCCCCCAGCCTGAATATGTG  
AACCAGCCAGATGTTTCGGCCCCAGCCCCCTTCGCCCCGAGAGGGCCCTCTGCCTGCT  
GCCCCGACCTGCTGGTGCCACTCTGGAAAGGGCCAAGACTCTCTCCCCAGGGAAGAAT  
GGGGTCGTCAAAGACGTTTTTTGCCTTTGGGGGTGCCGTGGAGAACCCCGAGTACTTG  
ACACCCCAGGGAGGAGCTGCCCCCTCAGCCCCACCCTCCTCCTGCCTTCAGCCCAGCC  
TTCGACAACCTCTATTACTGGGACCAGGACCCACCAGAGCGGGGGGCTCCACCCAGC  
ACCTTCAAAGGGACACCTACGGCAGAGAACCCAGAGTACCTGGGTCTGGACGTGCCA  
GTG SEQ ID NO: 5

## 1F. HER2 Polypeptide Sequence

MELAAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQ  
GNLELTYLPTNASLSFLQDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTOQLFEDNYAL  
AVLDNGDPLNNTTPVTGASPGGLRELQLRSLTEILKGGVLIQRNPQLCYQDTILWKD  
IFHKNNQLALTLLIDTNRSRACHPCSPMCKGSRGWGESSEDCQSLTRTVACAGGCARCK  
GPLPTDCCHEQCAAGCTGPKHSDCLACLFHNSGICELHCPALVTYNTDTFESMPNP  
EGRYTFGASCVTACPYNYLSTDVGSCTLVCPHLNQEVTAEDGTQRCEKCSKPCARVC  
YGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFLPESFDGDPASNTAPLQPEQLQV  
FETLEEITGYLYISAWPDSLPLDSVFNQLQVIRGRILHNGAYSLTLQGLGISWLGLR  
SLRELGSGLALIHNTLHLCFVHTVPWDQLFRNPHQALLHTANRPEDECVGEGLACHQ  
LCARGHCWGPPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHCLPCHPECQPQ  
NGSVTCFGPEADQCVACAHYKDPFFCVARCPSGVKPDLSPYMPIWKFPDEEGACQPCP  
INCTHSCVDLDDKGCPAEQRASPLTSIVSAVVGILLVVVLGVVFGILIKRRQKIRK  
YTMRRLLQETELVEPLTPSGAMPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIP  
DGENVKIPVAIKVLRENTSPKANKEILDEAYVMAGVGSPLYVSRLLGICLTSTVQLVT  
QLMPYGCLLDHVRENRRGLGSQDLLNWCMIKAGMSYLEDVRLVHRDLAARNVLVKS  
PNHVKITDFGLARLLDIDETEHADGGKVPIKWMALLESILRRRFTHQSDVWSYGVTV  
WELMTFGAKPYDGIPAREIPDLLEKGERLPQPPICTIDVYMIMVKCWMIDSECRPRF  
RELVSEFSRMARDPQRFVVIQNEDLGPASPLDSTFYRSLLLEDDDMGDLVDAEEYLVP  
QQGFFCPDPAPGAGGMVHHRHRSSTRSGGDLTLGLEPSEEEAPRSPLAPSEGAGS  
DVFDGDLGMGAAGLQSLPTHDPSPQLQRYSEDPTVPLPSETDGYVAPLTCSPQPEYV  
NQPDVRPQPPSPREGPLPAARPAGATLERAKTLSPGKNGVVKDVFAFGGAVENPEYL  
TPQGGAAPQPHPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVP  
V SEQ ID NO: 6